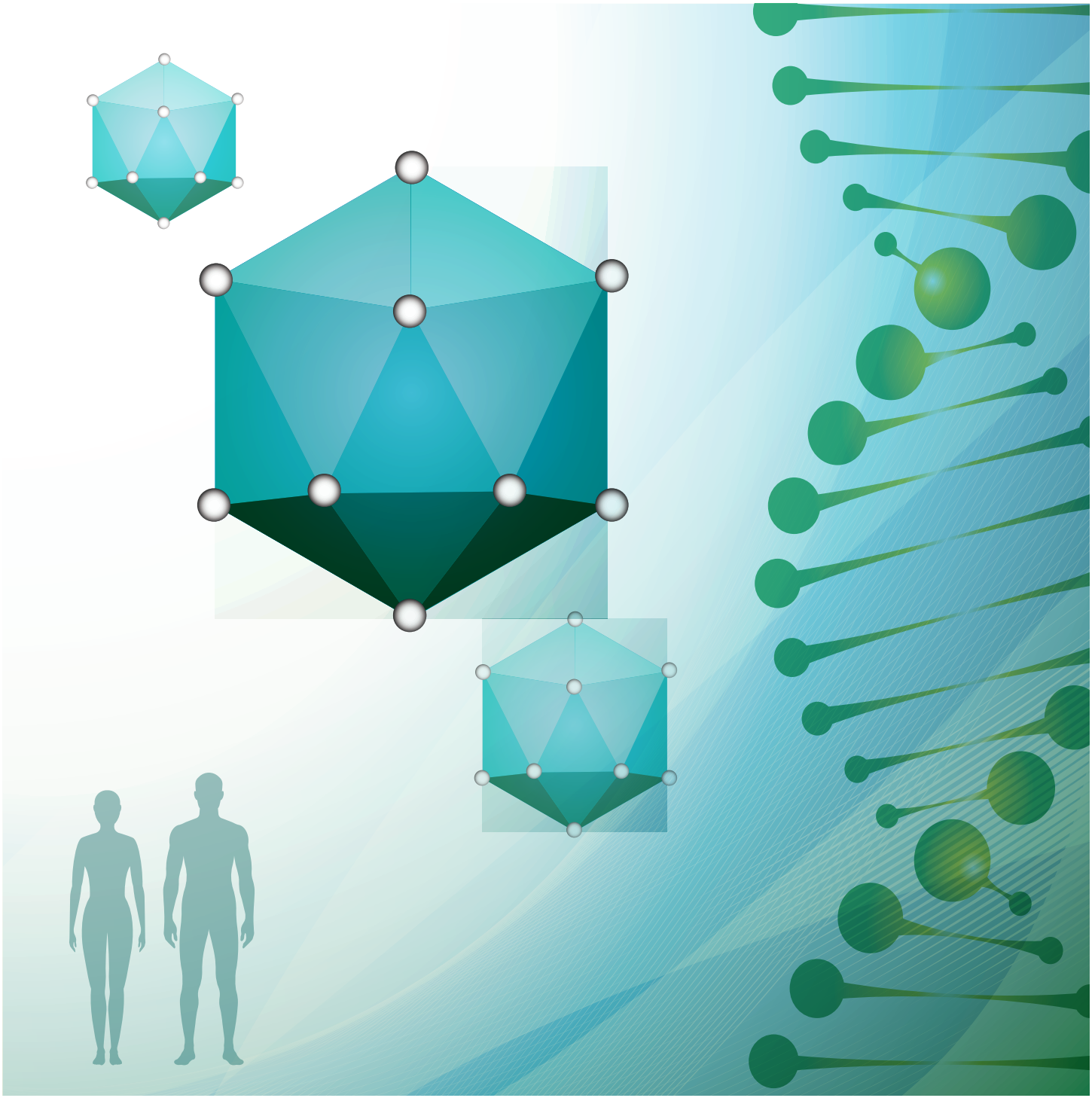
Therapeutic Development

Adeno-Associated Virus (AAV) Gene Therapy Applications and Solutions Guide



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BIO-RAD

OUR MISSION:
TO ADVANCE SCIENCE AND SAVE LIVES, TOGETHER

When you partner with us, we promise to provide high-quality products and services that advance scientific discovery and improve healthcare.

The journey of every therapeutic is unique. We offer a suite of customizable products and services that ensure efficiency, reproducibility, and reliability at each step of discovery, development, and manufacturing. You can rely on us, as a top-five global supplier of life science research and diagnostic tools, to provide platforms and solutions that perform so you can focus on moving science forward.

Solutions are manufactured in ISO 13485–certified production facilities, and our experienced global support team is available to provide technical and regulatory audit support.

AAV Gene Therapy
Key Application Map: Discovery to Release

Discovery
Viral Titer
Characterization
Potency

Preclinical and Clinical Research
Viral Titer
Characterization
Potency
Immunogenicity
Biodistribution and Viral Shedding

Process Development
Viral Titer
Characterization
Potency
Method Development
Residual Contaminants and Purity

Manufacturing and QC
Viral Titer
Characterization
Potency
Residual Contaminants and Purity

AAV VIRAL TITER

Accurately measuring viral titer is a key metric throughout gene therapy bioprocess development, whether you are in the early stage, scaling production, or running assays for quality control (QC) release. However, the stages of development may require different levels of analytical rigor or throughput.

Additional challenges arise due to AAV inverted terminal repeat (ITR) secondary structure and the difficulty of achieving acceptable amplification efficiency for use with traditional quantification methods. Regardless of your challenge or development stage, we have comprehensive PCR technology solutions to address your needs.

> **“ddPCR is the gold standard method to titer [AAV vector genomes].” (Alliance for Regenerative Medicine, 2021)**

Rapid Results — Real-Time PCR

In the early development of AAV gene therapies, sensitivity, dynamic range, and time to results are often criteria in developing initial titer assays.

When screening production cell lines or in process development, a large number of samples are generated requiring quick results to increase productivity and advance to the next stage.

Real-Time PCR (qPCR) has been established to provide the speed, throughput, and sensitivity required when these needs are most relevant.

CFX Opus Real-Time PCR System

QX ONE™ Droplet Digital™ PCR (ddPCR™) System

Increasing Precision — Droplet Digital PCR

As AAV therapies progress through development, the need for validated and highly reproducible assays increases. Also, achieving acceptable amplification efficiency using real-time PCR can be difficult due to the AAV genome structure, potentially leading to imprecise viral genome measurements. The endpoint PCR amplification used by ddPCR technology enables high accuracy and precision without the requirement for perfect amplification efficiency.

Bio-Rad’s family of qPCR and ddPCR instruments and validated assays provides a complete and scalable method to implement viral titer determination and characterization from discovery to QC.

VIRAL CHARACTERIZATION

Across the gene therapy development process, understanding the consistency of iterations of your AAV gene therapy is essential. Start with reliable tools to measure the physical properties of your viral vector, including capsid integrity, serotype identity, and genome integrity.

Stain-Free western blotting and SDS-PAGE are powerful tools to confirm serotype identity and capsid protein ratios, while Droplet Digital PCR enables high accuracy in assessing the AAV genome integrity.

Capsid Integrity and Serotype Identity

Routine characterization of AAV vectors includes analyzing their vector capsid protein ratios and validating the serotype identity. As more recombinant AAV vectors target different tissue types, it is important to validate these characteristics through development and into production.

Our Stain-Free technology offers the fastest method for checking capsid protein ratios to verify that the AAV viral particles are intact, and ChemiDoc™ MP Imaging System provides best-in-class sensitivity and flexibility across a full set of imaging methods.

ChemiDoc MP Imaging System

Genome Integrity

One common challenge is assessing the completeness of incorporated DNA packaging in vectors. By targeting multiple distinct regions of the viral genome with multiplex ddPCR technology, AAV vector genome integrity can be evaluated with high accuracy and precision. Custom ddPCR assays can be created with the ddPCR assay design tool, allowing rapid evaluation of novel AAV vectors.

This in-depth characterization can be used to optimize manufacturing and maximize potency.

QX600™ Droplet Digital PCR System

POTENCY

Potency tests establish the quality, stability, and strength of the product. Although regulatory agencies have not determined a preferred set of assays, multiple techniques are typically needed to provide complementary and accurate data.

This unique and product-dependent combination of assays is often developed from initial work performed in early-stage discovery and refined in preclinical and clinical research with the purpose of being used as lot-release testing upon approval.

> **“This one-step RT-ddPCR method simplifies the workflow, allows for duplexing reactions, and enables absolute quantification of transcripts without standard materials.” (Clarner et al. 2021)**

Target Persistence and Stability

Verifying that an AAV vector delivers sufficient and stable expression of the target protein is a common goal when protein replacement is the mechanism of action. Western blotting is a fast and flexible tool to measure protein expression across cell lines, tissue types, and time points. This can be challenging, as any one reference protein may exhibit varying signal levels across these samples. Using a total protein signal to serve as a reference enables reliable quantification and normalization in these experiments.

The Stain-Free Western Blotting Workflow easily facilitates total protein normalization (TPN) to allow a flexible reference signal with minimal steps.

Stain-Free Western Blotting

Infectivity

Infectivity and transduction efficiency of AAV vectors are often measured using one-step reverse transcription Droplet Digital PCR (RT-ddPCR) methods when precision and accuracy are critical, as Droplet Digital PCR offers a reduced coefficient of variance (CV) regardless of testing site or operator. Conversely, reverse transcription quantitative PCR (RT-qPCR) is used if sample throughput or faster time to results are desired.

Regardless of your challenge, Bio-Rad offers the quality and reliable solutions you have come to count on to address your needs.

PCR Instruments Portfolio

IMMUNOGENICITY

While validating the effects of your AAV vector, it is important to assess the immune response both toward the vector and any process-related impurities such as host cell protein or DNA/RNA. Potential immunogenicity is a common safety concern; therefore, avoiding severe immune reactions at the time of therapeutic infusion is a top priority.

By monitoring the immune response in preclinical and clinical stages, it allows characterization of the therapeutic effect and observation of both target and off target activation, i.e., immunotoxicity.

> **Immunogenicity and cellular toxicity are critical considerations when assessing the potential efficacy of AAV therapeutic approaches.**

Multiplex Immunoassays

When analyzing the immunogenicity and immunotoxicity of your AAV gene therapy, Bio-Plex™ Pro Cytokine and Chemokine Panels provide high sensitivity across the physiologically relevant ranges, ensuring detection of these important immunoproteins. These multiplex assays also provide a high-throughput workflow, allowing characterization of the immune profile with low sample input. Choose from fixed multiplex panels or customize assays for your experimental requirements.

Bio-Plex Pro Cytokine Panel

Phenotypic Assays

Monitoring immune responses across individuals or time points often involves analyzing numerous samples. These can be varied in terms of complexity, throughput, and cell type used. Assays that investigate more complex cellular responses such as those involved in immunogenicity require high numbers of cells and ten or more parameters.

The ZE5 Cell Analyzer stands out with advanced multiparameter capabilities, efficiently processing high sample volumes with walk-away automation. This facilitates rapid evaluation of cellular immune profiles and accurate comparison of responses across samples.

ZE5 Cell Analyzer

BIODISTRIBUTION & VIRAL SHEDDING

To accurately measure biodistribution, detection of both the vector and the transgene protein is recommended. This allows collection of correlative evidence showing the presence, persistence, activity, and effect of the AAV vector. In addition, viral shedding is monitored to assess the clearance of the AAV gene therapy product. AAV vectors may be present at vanishingly low levels in some tissues, necessitating highly sensitive detection techniques.

> **“The endpoint assay in ddPCR minimizes the matrix effect, facilitating preparation of a single surrogate calibration curve in combination with external control gene and DNA recovery normalization, which is superior to the qPCR-based method in terms of animal ethics and cost.” (Nakayama et al. 2023)**

Established and Reliable — Real-Time PCR

Real-time PCR is often considered ideal for biodistribution and viral shedding assays because of its wide dynamic range, high throughput, and speed (Ma et al. 2020). A well-designed assay in combination with a thorough sample prep method can meet the regulatory LLOD guideline of 50 copies/g of human genomic DNA.

The CFX Opus Real-Time PCR System offers reliability, sensitivity, and multiplexing capability, along with user-intuitive software with regulatory compliance–enabling capabilities. From flexibility of platform choice to automation incorporation, the CFX Opus platform can assist your biodistribution studies.

QX600 Droplet Digital PCR System

CFX Opus Real-Time PCR System

Expanding Sensitivity — Digital PCR

Droplet Digital PCR has been shown to be more reproducible and sensitive for rare targets in limited samples (Nazir 2023). It also has a higher tolerance for inhibitors, as it does not depend on amplification efficiency for quantification.

The use of digital PCR is on the rise in the pharmacology phases of therapeutic development, especially in cases where assay sensitivity requirements are not being met with conventional methods.

METHOD DEVELOPMENT

While characterization and analytical assays are critical to AAV lot release, downstream method development and process optimization for the capture and polishing steps are essential to obtain the yield, purity, and process economics necessary. Bio-Rad has a dynamic portfolio of chromatography instrumentation, prepacked and empty columns, media, and resins that can be utilized for AAV development and manufacturing.

Our team of scientists is here to assist you with purification method development from bench to pilot scale through process transfer to manufacturing.
Contact our scientists today to accelerate your process development.

Process Optimization

From initial resin screening to process development through production and manufacturing scale, Bio-Rad has a wide range of tools in multiple formats to assist in downstream purification. Our chromatography resins enable straightforward process optimization to deliver both high purity and yield. From plate-based resin screening to good manufacturing practice (GMP) ready prepacked columns, we have solutions for your purification needs. Contact us for samples today.

Bioprocess Chromatography Products

Method Optimization

Our NGC Chromatography System is the perfect companion for lab-scale purification and method development. The customizable design allows the system to be upgraded to provide higher flow rates, sophisticated detection capabilities, pH monitoring, column or resin scouting, and multidimensional chromatography.

NGC Chromatography System

RESIDUAL CONTAMINANTS & PURITY

Impurities and contaminants in AAV products can cause adverse responses upon administration. Therefore, purity of the viral vector must be confirmed prior to administration in both preclinical and clinical trials, as well as throughout the manufacturing QC process. To ensure patient safety, purity analysis requires a multitude of tests using techniques that measure host cell proteins (HCP), host cell DNA (HC DNA), and common contaminants such as *Mycoplasma*.

Host Cell Proteins

When you are part of a team working in the early stages of viral vector development, SDS-PAGE analysis is a quick and relatively inexpensive way to check for the presence of nonviral proteins in your prep. While conventional fluorescent protein stains, such as SYPRO Ruby, are highly sensitive, they can slow you down with their overnight protocols.

Bio-Rad's Stain-Free imaging enables detection of contaminating proteins in minutes rather than overnight, for rapid assessment of protein purity when your priority is speed.

Stain-Free Imaging

Mycoplasma

The probe-based chemistry, Vericheck ddPCR *Mycoplasma* Detection Kit brings confidence to *Mycoplasma* contamination testing. The specificity and reproducibility of this kit allows for a precise measurement of up to 112 *Mycoplasma* species using Droplet Digital PCR.

Vericheck ddPCR *Mycoplasma* Detection Kit

Host Cell DNA

The standard methods used for host cell DNA quantification can face challenges due to lack of specificity and reproducibility. Vericheck ddPCR HEK293 Kits feature novel ddPCR assays that have been designed to quantify residual HEK293 DNA and its size with high specificity and reproducibility without a reference curve.

Vericheck ddPCR HEK 293 Residual DNA Quantification Kit

Vericheck ddPCR HEK 293 Residual DNA Size Kit

SUPPORT, SOFTWARE, & COMPLIANCE

From hardware and instruments to consumables and software with tools to assist with compliance, we are ready to partner with you to address your needs in the AAV gene therapy space.

Bio-Rad offers software editions enabling traceability features that facilitate compliance with regulations such as U.S. FDA 21 CFR Part 11.

Support

With more than 50 years of dependable service supporting the scientific community, we are here when you need us.

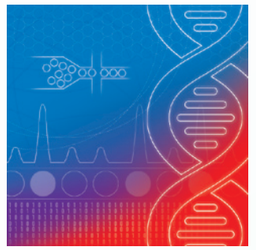
Ensure coverage for routine maintenance and emergency repairs at no additional cost with a Bio-Rad service contract plan. Plans include:

- Service and repair coverage
- Replacement parts, travel, and labor
- Prioritized response time
- Preventative maintenance

For regulated environments, equipment qualification services are available. Services include:

- Installation Qualification (IQ)
- Operational Qualification (OQ)
- Preventative Maintenance (PM)
- Thermal validation

Software



QX Manager Software
Supports QX200™ and QX600 Droplet Digital PCR Systems

QX ONE Software
Supports QX ONE Droplet Digital PCR System

CFX Maestro Software
Supports CFX Real-Time PCR Systems

ChromLab Software
Supports NGC Chromatography Systems

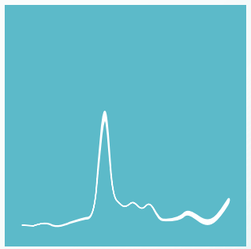
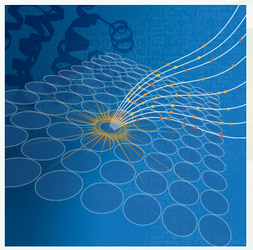


Image Lab Software
Supports GelDoc™ Go and ChemiDoc Imagers, GS-900 Densitometer

Everest Software
Supports ZE5 Cell Analyzer



Bio-Plex Manager Software
Supports Bio-Plex 200 System

		Applications						
Technique	Key Products	Viral Titer	Viral Characterization	Potency	Immunogenicity	Biodistribution	Method Development	Residual Contaminants & Purity
Digital PCR	QX200 System	X	X	X		X	X	X
	QX600 System	X	X	X		X	X	X
	QX ONE System	X	X	X		X	X	X
	Assays	X	X	X		X	X	X
Imaging	ChemiDoc System		X	X			X	X
	ChemiDoc MP System		X	X			X	X
	Gels and blots		X	X			X	X
	Trans-Blot™ Turbo System		X	X			X	X
Real-time PCR	CFX Opus 96 System	X		X		X	X	X
	CFX Opus 384 System	X		X		X	X	X
	Assays	X		X		X	X	X
	Reagents and consumables	X		X		X	X	X
Chromatography	NGC Chromatography System						X	
	Screening tools						X	
	Prepacked columns						X	
	Process resins						X	
	Analytical columns							X
Flow cytometry	ZE5 Cell Analyzer				X			
	Panels				X			
	Antibodies				X			
Multiplex immunoassay	Bio-Plex 200 System				X			
	Bio-Plex 3D System				X			
	Bio-Plex Pro Wash Station				X			
	Assays				X			
	Raw beads and coupling reagents				X			

References

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